

α -subunit of Nav1.2, using whole cell patch clamp techniques. Voltage protocols were run with extracellular solutions of pH 7.4 and pH 6.0 before and after the addition of ranolazine. The addition of 100 μ M ranolazine at pH 7.4 led to a significant decrease in late sodium current, faster rate of open state fast inactivation, and slower recovery from inactivation. Similar trends were seen in preliminary experiments at 10 μ M and 30 μ M ranolazine. In addition, we observed a tonic block of peak current and an increase in total use-dependent block. Many of these effects were different at low pH. Low pH led to a significant depolarizing shift of the conductance curve, and slowed the onset of fast inactivation. Adding ranolazine at pH 6.0 significantly decreased the rate of fast inactivation recovery, and increased the total use-dependent block and rate of open state inactivation. Although directions of the effects were unchanged, the magnitude of these effects was significantly different between the two pH values. Our results suggest that ranolazine stabilizes fast inactivation at both pH 7.4, and pH 6.0. It is possible that ranolazine and protons may compete for a common binding site or indirectly interact leading to decreased ranolazine efficacy at low pH. (Supported by a research grant from Gilead Sciences, Inc. and an NSERC Discovery Grant to PCR, and an NSERC URSA to CHP.)

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Contribution of Local Anesthetic Binding Site Residues F1760 and Y1767 to Block of the Cardiac Na⁺ Channel, hNav1.5, by Ranolazine

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Amino acid residues F1760 and Y1767 in S6/Domain IV of voltage gated Na⁺ channels are part of the local anesthetic binding site in the inner mouth of the pore. Mutagenesis experiments have shown that use-dependent block (UDB) of the cardiac hNav1.5 Na⁺ channel by the Class Ia anti-arrhythmic drug lidocaine is significantly reduced by alanine substitutions at these two sites. The antianginal drug ranolazine, which has structural similarity to lidocaine, was shown previously to block the cardiac late sodium current (I_{Na}) and to block peak and late I_{Na} in a use-dependent manner (Rajamani et al., 2009). As with lidocaine, UDB of peak I_{Na} by ranolazine was reduced in the F1760A mutant channel (Fredj et al., 2006; Wang et al., 2008). We used the F1760A and Y1767A mutants to investigate further the block by ranolazine of the closed, inactivated, and open states of the channel. To measure affinity to the open state, we used an inactivation-deficient hNav1.5 channel (L409C/A410W, Edrich et al., 2005) in the presence and absence of the F1760A or Y1767A mutations. We found that both mutations (F1760A and Y1767A) significantly decreased UDB of peak I_{Na}, block of the open state, and block of late I_{Na} by ranolazine compared to WT channels (14-24 fold shift of the value of IC₅₀ for ranolazine). The effect of the mutations on block by ranolazine of the resting and inactivated states was modest (1.3-1.9 fold shift of the value of IC₅₀ for ranolazine). The results indicate that both the F1760A and Y1767A mutations reduce the effect of ranolazine to cause UDB and open state block of hNav1.5.

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Using Full Factorial Design of Experiments on an Automated Patch Clamp Instrument: A Case Study on the Ionworks Quattro and Nav1.5 Channel

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In the past decade, technological advancements have enabled automation of patch clamp recordings. Instruments like the Ionworks Quattro produces electrophysiological data from a 384 well plate in about an hour. However, they also require proprietary and expensive one-use consumables like the Patch-Plate. Conversely, the large amount of data produced from these instruments also allows more than the "one factor at a time" experiments. Here we present an example of a full factorial experiment on the Nav1.5 channel using the Ionworks Quattro. A full factorial experimental design provides more information about the instrument by having a more precise estimation of each factor alone and by estimating the interactions between factors.

We tested the potencies of several known blockers on the cardiac sodium channel (Nav1.5) and varied 3 factors: incubation time, mixing cycles, and cell density. From the experiment, we find that the IC₅₀s of most compounds are affected by incubation time and cell density. We also observed interactions with the factors of incubation time/cell density and the number of mixing cycles/cell density. From this case study, we propose that a factorial design of experiments can be done effectively with an automated patch clamp instrument to optimize assay conditions with enriched information to the effect of reduction of consumables and shortened time of assay development.

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Functional Studies of Interaction Between Huwentoxin-IV and Voltage-Gated Sodium Channel Nav1.7

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Voltage-gated sodium channel 1.7 (Nav1.7) plays a role in the conduction of action potentials and is involved in the sensation of pain. Spider toxins such as Huwentoxin IV (HwTx-IV) are potent inhibitors of Nav1.7, functioning as a gating modifier trapping the voltage sensor in an inward closed conformation. HwTx-IV interacts with specific residues in the voltage sensor S3-S4 region of domain II. The purpose of the present study was to understand the residues important for interaction between HwTx-IV and Nav1.7. Computationally, molecular dynamics was used to study wild type HwTx-IV along with various alanine and cysteine mutations to determine residues important for the stability of toxin and to obtain a model of the interacting surface. A homology model of Nav1.7 was built and the toxin docked to determine key interactions. Experimentally, alanine mutants of HwTx-IV were tested for functional activity using FLIPR, QPatch, and manual patch. Computational and experimental results suggest key residues critical for binding of the toxin to Nav1.7, particularly S25, W30 and K32. These studies provide a more clear idea of the nature of interaction between HwTx-IV and Nav1.7 and may therefore be a useful guide in designing novel peptides with improved selectivity for sodium channel subtypes.

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"Clip Detecting" with Series Resistance Compensation using an Automated Patch Clamp System

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One of the significant challenges for automated patch clamp has been to incorporate amplifiers with the ability of adjusting to changes in membrane capacitance, series resistance (Rs) and voltage-clamp time constants without user intervention. If these adjustments are not made correctly there is a risk of generating un-precise data or even losing data due to oscillations in the recorded trace. Rs compensation capability of the patch clamp amplifier of QPatch is presented. A new "clip detector feature" allowing Rs to be temporarily turned off avoiding loss of cells due to fatal oscillations, and thereby increasing the data throughput while maintaining high quality recordings, is presented. Clamping whole-cells can be a challenge depending on the magnitude of Rs. The series resistance produces two undesired effect on whole cell voltage clamp recordings: i) The cells not being clamped to the desired potential due to the voltage drop over the Rs ($V_{\text{drop}} = R_s \times I$), ii) The resolution of the voltage clamp in fast physiological processes being affected by the Rs. It is essential to reach adequate series compensation in a timely manner to obtain accurate recordings. As such, the quality of data depends on the quality of the patch clamp amplifier and its features. In order to make fast 100% series resistance compensation a fast patch clamp amplifier is needed. The embedded amplifier in the QPatch utilizes a patented technique invented by Adam Sherman (Alembic Instruments). QPatch data from human SK potassium and fast sodium channels are shown. The data demonstrates that i) QPatch measures the fastest sodium channels with accurate clamp, and ii) the patch clamp amplifier in QPatch has the required speed to record the correct current amplitude when the current through an open SK channel is reversed.

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Inhibition of Voltage-Gated Na⁺ Currents in Sensory Neurons by the Sea Anemone Toxin APETx2

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ASICs are neuronal H⁺-gated channel that are transiently opened by extracellular acidification. Functional ASIC channels are made of homotrimeric or heterotrimeric complexes of different ASIC subunits (ASIC1a, -1b, -2a, -2b and 3). APETx2, a toxin from the sea anemone *Anthopleura elegantissima* inhibits homotrimeric ASIC3 with IC₅₀ < 100 nM. In rat sensory neurons (DRG), where heterotrimeric channels are expressed, higher concentration are necessary to block ASIC3 containing channels (0.1-2 μ M). Several animal studies relied on APETx2 as a selective pharmacological tool to study ASIC3 physiological role.